

WHAT IS CLAIMED IS:

1. A method of determining a binding capacity of a surface, the method comprising:
 - providing the surface containing a reactive moiety;
 - 5 providing a fluorophore comprising a fluorescent moiety adapted to emit a detectable signal;
 - reacting the fluorophore with the reactive moiety to form a linking bond between the fluorophore and the reactive moiety;
 - cleaving a cleavable bond to liberate the fluorescent moiety; and
 - 10 detecting the detectable signal to determine the binding capacity of the surface.
2. A method of determining a binding capacity of a surface, the method comprising:
 - providing the surface containing a reactive moiety;
 - providing a fluorophore comprising a fluorescent moiety adapted to emit a detectable signal;
 - 15 reacting the fluorophore with the reactive moiety to form a linking bond between the fluorophore and the reactive moiety, wherein the linking bond is the cleavable bond and is a disulfide bond or an aromatic azo group;
 - cleaving a cleavable bond to liberate the fluorescent moiety; and
 - detecting the detectable signal to determine the binding capacity of the surface.
- 20 | 3. The method of claim 2, wherein the cleavable bond is a disulfide bond.
4. The method of claim 2, wherein the aromatic azo group is represented by a formula:



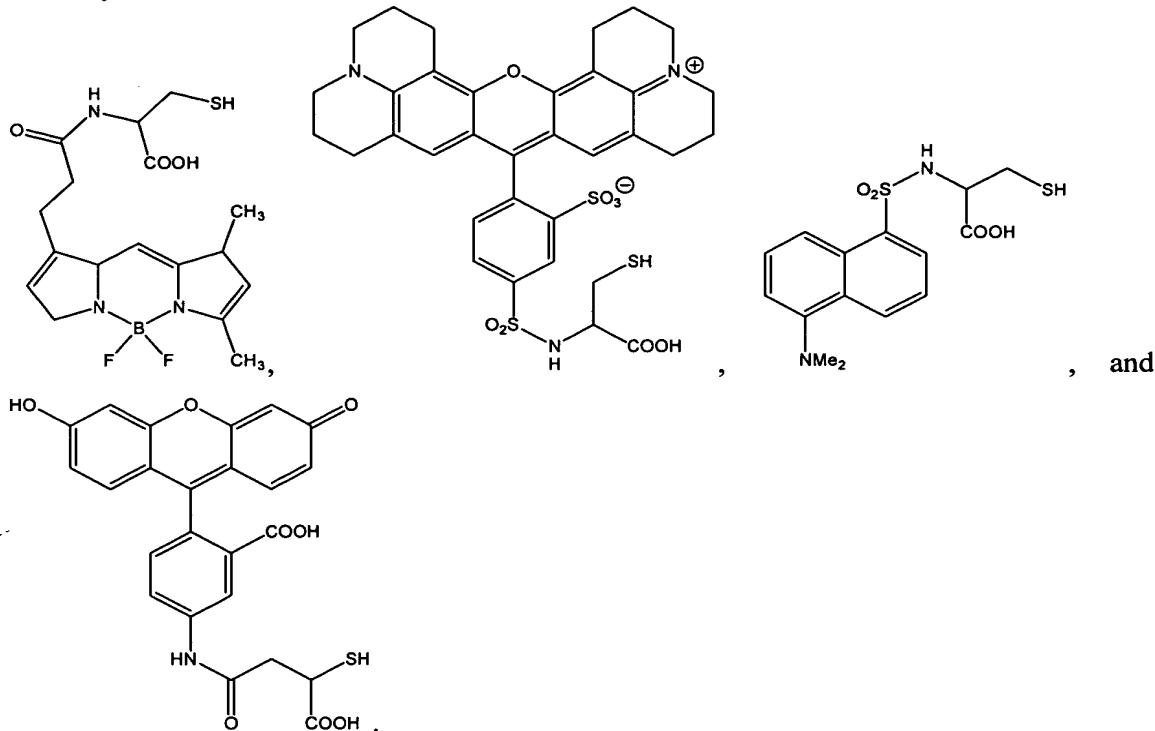
wherein R^2 is an aromatic compound selected from the group consisting of a heterocyclic group and an electron-deficient aromatic group.

25 5. The method of claim 2, wherein the fluorophore is a thiol-containing fluorescent structure represented by a formula:

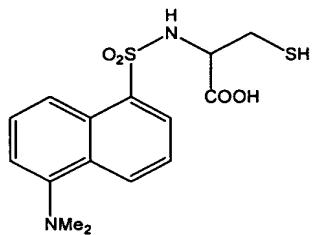


wherein Fl is the fluorescent moiety and is a member selected from the group consisting of fluorescent L-cysteine, BODIPY-L-cysteine, fluorescein and derivatives thereof.

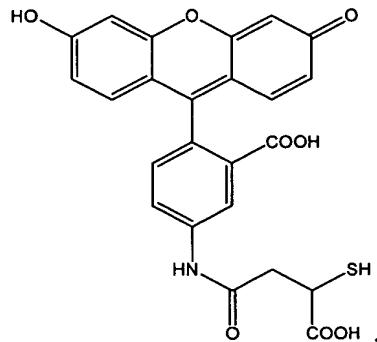
30 6. The method of claim 5, wherein the thiol-containing fluorescent structure is a member selected from the group consisting of:



7. The method of claim 5, wherein the thiol-containing fluorescent structure is



5 8. The method of claim 5, wherein the thiol-containing fluorescent structure is:



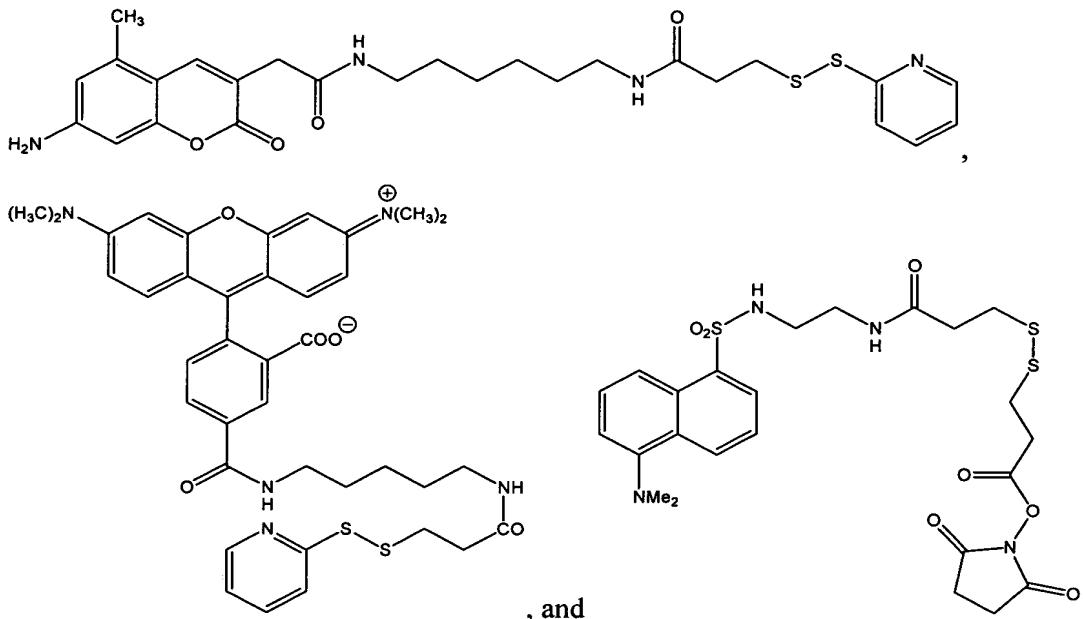
9. The method of claim 2, wherein the fluorophore is a thiol-reactive fluorescent structure represented by a formula:

Fl-S-X

wherein X is a member selected from the group consisting of Cl, SO₃(C₁-C₆ alkyl), and S-R², wherein R² is a heterocyclic group or an electron-deficient aromatic group.

10. The method of claim 9, wherein R is a pyridyl group or a phenyl group substituted with one or more electron-withdrawing substituents.

5 11. The method of claim 9, wherein the thiol-reactive fluorescent structure is a member selected from the group consisting of:



12. The method of claim 2, wherein the fluorophore further comprises a functional group, wherein the functional group is bound to the fluorescent moiety by the cleavable bond and is reacted with the reactive moiety to form an uncleavable bond such that cleaving predominantly occurs at the cleavable bond.

10 13. The method of claim 12, wherein the functional group is a member selected from the group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.

15 14. The method of claim 2, wherein the surface is a member selected from the group consisting of a polymer, a metal, a biomaterial, a ceramic, and a semiconductor.

15. The method of claim 14, wherein the polymer is polyurethane.

16. The method of claim 2, wherein the reactive moiety is a thiol, a thiol-reactive group or a group adapted to be converted into a thiol or a thiol-reactive group.

20 17. The method of claim 2, wherein the reactive moiety is a thiol group or an amino group.

18. The method of claim 17, wherein the reactive moiety is further reacted with 5,5'-dithio-bis(2-nitrobenzoic acid) or succinimidyl 3-(2-pyridyldithio)propionate.

19. The method of claim 2, wherein the reactive moiety is a dithio group.

20. The method of claim 2, wherein the cleavable bond is cleaved by using a
5 reducing agent selected from the group consisting of dithiothreitol, β -mercaptoethanol, mercaptoethylamine hydrochloride, a borohydride, and a phosphine.

21. The method of claim 20, wherein the borohydride is sodium borohydride.

22. The method of claim 20, wherein the phosphine is a member selected from the
group 10 consisting of tris(2-cyanoethyl)phosphine, tris(2-carboxyethyl)phosphine and trimethylphosphine.

23. A kit for practicing of method of claim 2, the kit comprising a fluorophore.

24. The kit of claim 23, wherein the fluorophore comprises the fluorescent moiety
and a linking bond precursor.

25. The kit of claim 23, wherein the linking bond precursor is adapted to form a
15 cleavable disulfide bond or an aromatic azo group.

26. The kit of claim 25, wherein the linking bond precursor is $-SH$.

27. The kit of claim 25, wherein the linking bond precursor is represented by a
formula:



20 wherein X is a member selected from the group consisting of Cl , $SO_3(C_1-C_6\text{ alkyl})$, and $S-R^2$,
wherein R^2 is a heterocyclic group or an electron-deficient aromatic group ...

28. The kit of claim 23, wherein the fluorophore further comprises a functional
group, wherein the functional group is bound to the fluorescent moiety by the cleavable bond
and is adapted to react with the reactive moiety to form an uncleavable bond.

25 29. The kit of claim 28, wherein the functional group is a member selected from the
group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.

30. The kit of claim 28, wherein the uncleavable bond is an amide bond.